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BACTERIA OF PUBLIC HEALTH SIGNIFICANCE ASSOCIATED WITH FISH REARED IN TREATED WASTEWATER

by

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The suitability of tertiary wastewater ponds for aquaculture was evaluated in terms of fish productions obtained and the significance of bacteria in the wastewater and from fish. Silver carp (Hypothalmichthyes molitrix) were stocked into tertiary wastewater ponds at 10,000 fish per hectare and cultured five months. Fish growth was determined monthly. Bacterial evaluations of water and fish were made monthly. Pour plates of the appropriate media were made to determine total plate count, fecal coliforms and fecal streptococci. Enrichment procedures were used to isolate Salmonella. The relative abundance of selected bacterial colony types was found over the course of the experiment. Selected isolates were identified. Net production of fish was 1,309.2 kg/ha with the average weight of fish produced being 204.6 grams. Fecal coliforms and fecal streptococci were commonly found from both water and fish. Salmonella was rare in the wastewater ponds and very rare from the fish. Changes over time in fecal coliform or fecal streptococci from water generally were not represented by a corresponding response for that bacterial count from fish. Corresponding trends were more evident in total counts from water and fish. Certain colony types were much more common in water than fish, while others were more common from fish. Klebsiella pneumoniae was the most common fecal coliform from the water while Aeromonas hydrophila was the most common from fish. Streptococcus faecalis was the most common fecal streptococcus from both water and fish.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH conducts a coordinated environmental health research program in toxicology, epidemiology and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides and non-ionizing radiation. The laboratory participants in and development and revision of air and water quality criteria and health assessment documents on polutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare for all U.S. inhabitants.

An area of study supported by the Health Effects Laboratory is the utilization of wastewater and the environmental consequences of such utilization. Aquaculture is potentially a very effective utilization of wastewater. Emphasis can be given to aquaculture systems that have had their primary goal of improving water quality or systems directed toward production with improvement in water quality being secondary. In either case an organism is being produced in a wastewater environment that should be harvested and utilized. How that production is utilized depends on a variety of factors including public health implications.

Various studies have found that fish from contaminated natural waters may harbor pathogenic bacteria. The significance of bacteria of public health importance in tertiary wastewater lagoons and their relationship to the bacterial flora of fish in that environment is not well established and was the focus of this study.

F. G. Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

The suitability of tertiary wastewater ponds for aquaculture was evaluated in terms of fish productions obtained and the significance of bacteria in the wastewater and from fish. Silver carp Hypothalmichthyes molitrix were stocked into tertiary wastewater ponds at 10,000 fish per hectare and cultured five months. Fish growth was determined monthly. Bacterial evaluations of water and fish were made monthly. Pour plates of the appropriate media were made to determine total plate count, fecal coliforms and fecal streptococci. Enrichment procedures were used to isolate Salmonella. The relative abundance of selected bacterial colony types was found over the course of the experiment. Selected isolates were identified.

Net production of fish was 1,309.2 kg/ha with the average weight of fish produced being 204.6 grams. Fecal coliforms and fecal streptococci were commonly found from both the water and fish. Salmonella was rare in the wastewater ponds and very rare from the fish. Changes over time in fecal coliform or fecal streptococci from water generally were not represented by a corresponding response for that bacterial count from fish. Corresponding trends were more evident in total counts from water and fish. Certain colony types were much more common in water than fish, while others were more common from fish. Klebsiella pneumoniae was the most common fecal coliform from the water while Aeromonas hydrophila was the most common from fish. Streptococcus faecalis was the most common fecal streptococcus from both water and fish.

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SECTION 1

INTRODUCTION

Aquaculture is an effective way to provide quality protein. It is taking on a more important role in the world food supply as the traditional capture fisheries are reaching their production limits, while the human population continues to grow. In the U.S. aquaculture is a rapidly growing industry meeting the American consumers demand for more lean, nutritious meats.

The basic objectives of aquaculture are to provide a suitable environment for the growth of the organism and produce an acceptable product. To meet these objectives requires an adequate availability of land, water and nutrients, and culture systems that will produce an acceptable product. The competition in the U.S. for land, water and nutrients has resulted in culture systems with high input costs aimed at producing luxury products. Where input costs can be lowered other aquaculture systems can be developed to produce products for other markets.

A rich source of water and nutrients which is going underutilized in the U.S. is domestic wastewater. The use of this water would allow the development of aquaculture systems that otherwise would not be practical. An appropriate use of wastewater is the culture of filter feeding fishes in tertiary wastewater lagoons. Tertiary wastewater lagoons are rich environments for aquaculture with an abundance of natural food organisms as a result of the natural food chain based on photosynthesis. The wastewater held in ponds is further purified through biological action while at the same time stimulating the production of algae and other food organisms. Planktonic algae are the primary producers in a wastewater pond. Other organisms feed on this production and pass this energy on through the food chain. Each time that energy is passed through the food

chain there is a significant loss in efficiency. Ideally in aquaculture a desirable organism can be produced that takes direct advantage of the primary production without having to pass energy several steps up thus increasing the efficiency of the system by reducing the steps in the food chain.

There are several species of fish which can utilize directly the primary production of planktonic algae in wastewater ponds. A species showing a great deal of promise is the silver carp Hypopthalmichthyes molitrix. It is a filter feeding fish with very closely set gill rakers that form a matrix capable of removing 8 um algae and other suspended matter from the water column. It has the potential for rapid growth and able to convert a less usable biomass, i.e., algae into a more usable one, i.e., fish.

The culture of silver carp or other fishes in wastewater could become widespread if the product were considered acceptable. A major factor in determining whether such a product is acceptable and to what use it could be put, is what public health dangers might exist. These dangers could include the presence of significant amounts of pathogenic viruses, bacteria, parasites, or toxic chemicals. Secondarily treated domestic wastewater will contain low levels of human pathogens and toxic elements. But the presence of these agents in the water supply or the pond environment does not necessarily imply that aquaculture organisms produced in that environment would contain these agents in hazardous levels or not be of acceptable quality.

One of the indices of the quality of a product is the types and abundance of bacteria associated with the product. The bacterial flora of fish is often thought to be a reflection of that of the environment. In a domestic wastewater pond that environment would include human fecal bacteria. The degree in which

silver carp reared in wastewater are a reflection of the bacteria in the water column will strongly influence how the fish produced from such an environment can be used.

This study focused on the culture of silver carp, their bacterial flora and how it reflects the bacterial flora of the environment. Several issues were addressed: the suitability of ponds receiving secondarily treated wastewater for the culture of silver carp; the nature of the bacterial flora of the water and fish and their interactions; antibody production by silver carp; the effect of exposing silver carp to water containing Salmonella; and a comparison of bacterial flora before and after processing from wastewater and non-wastewater cultured fish.

SECTION 2

CONCLUSIONS

Tertiary wastewater lagoons are good environments for the culture of silver carp. The fish grew well and were able to tolerate the environmental stresses encountered. No disease outbreaks among the fish occurred although such fish pathogens as Aeromonas were present.

Fecal coliforms, fecal streptococci and Salmonella were present from fish cultured in the wastewater environment. The levels of bacteria were similar to those reported from domestic livestock with the exception of fecal coliforms which were higher. Aeromonas, a saprophytic water bacteria, was a common component of the fecal coliform counts from fish. Thus the fecal coliform counts were distorted from the more classically considered components. Bacterial counts from both wastewater and non-wastewater cultured fish were similar after the fish had been processed. The levels found were similar to those reported for other forms of processed meats.

Silver carp are capable of being carriers of Salmonella when experimentally exposed to high concentrations in the water column. The fish can retain Salmonella in the intestine for 15 days after the fish are transferred to freshwater. Salmonella was rare from the fish cultured in the wastewater and was confined to the intestine.

In general the levels of bacteria encountered in this study were within the range of levels encountered from commercially sold fish. The absence of Salmonella from the mucous or flesh, after processing of fish which carried Salmonella in the intestine, suggests that such fish could be successfully processed for consumption.

The relationship of total plate count, fecal coliform and fecal streptococci from the wastewater and the fish are not simple. Bacterial counts from influent waters had little relationship to those from fish. Total plate counts of mid and effluent waters had some relationship to mucous and intestine counts of fish. This was not apparent for the fecal coliform or fecal streptococci. Various genera of bacteria would be present at more than one site, but its relative abundance would be different. Data obtained in this study would suggest that the bacterial flora of fish is not a good reflection of its aquatic environment.

SECTION 3

RECOMMENDATIONS

A clear set of standards needs to be developed to establish what fish, irrelevant of the environment from which they came, are suitable for human consumption. The levels of indicator organisms found in this study are similar to those of fish from other environments. The suitability of indicator groupings, i.e., fecal coliform and fecal streptococci as criteria for evaluating fish quality should be determined. Results of this study indicate that fecal coliform counts from fish can be misleading due to the frequency in which Aeromonas is found. Similarly fecal streptococci counts from fish may be distorted by corynebacterium-like organisms. Specific pathogenic bacteria need to be identified as target organisms in determining the suitability of fisheries products for consumption.

The significance of Salmonella in regard to producing fish in wastewater needs further study. Salmonella can be retained in the intestine of fish for a length of time but is rarely found from the body surface mucous even after processing. Further study of non-wastewater cultured fish, the occurrence of Salmonella in the intestine and its occurrance in surface mucous and flesh, is needed before conclusions can be made concerning wastewater cultured fish and Salmonella.

The presence or absence of pathogenic bacteria should only be one criterion in determining the suitability of wastewater cultured fish for consumption. The significance of pathogenic viruses and parasites, as well as toxic chemicals needs to be determined. An adequate base line of information from non-wastewater cultured fish needs to be established before conclusions can be made regarding the occurrence of toxins or pathogens in wastewater cultured fish.

This study, as have others, showed that wastewater is suitable for fish culture. What has not been adequately addressed is whether fish culture is a desirable wastewater treatment system. Silver carp are able to remove quantities of algae from a wastewater lagoon. But what density of fish is required to make a wastewater lagoon a stable treatment system is unknown. Controlled experiments are needed to determine the value of silver carp in wastewater treatment.

SECTION 4

EXPERIMENTAL PROCEDURES

FISH PRODUCTION

The production potential of silver carp was determined in earthen ponds receiving secondarily treated domestic wastewater. Three 400 m^2 ponds were stocked with silver carp at 10,000 per hectare. The water supply was a continuous gravity flow of secondarily treated wastewater from the final clarifier of the South Sewage Treatment Plant of the City of Auburn, AL. This plant is a trickling filter system plant which receives domestic sewage and small amounts of light industry wastewater. Each pond had its separate inlet and outlet with the flow rate adjusted to give a retention time of approximately seven days. Daily records of dissolved oxygen and temperature were made from the surface water. Afternoon measurements of dissolved oxygen, temperature and pH were made weekly in the latter part of the study. Fish mortalities and other observations were recorded daily.

The fish were first stocked in February of 1983 but a flood one month later necessitated that an inventory be made and stocking rates be readjusted to give a rate of 10,000 fish per hectare with an average weight of 21.22 g per fish. The fish were then cultured from March to August of 1983. During that time monthly samples were made to determine fish growth. The samples were taken using a 15 m seine and a 10% subsample weighed. Ten fish from each pond were removed at that time, anesthetized with 10 mg/l of quinaldine then taken in water from the same pond to the laboratory for bacteriological examination.

WATER SAMPLES

Bacteriological examinations, of influent and effluent waters and a midwater sample taken in the center of the pond 25 cm below the water surface, were made monthly beginning in January from each pond. These samples were taken

back to the laboratory, refrigerated and processed within three hours. Serial dilutions were made using phosphate-buffered saline. Two days prior to each monthly sampling, water samples were processed to determine appropriate dilutions of samples to give countable plates.

Standard plate counts and fecal streptococci were enumerated following APHA procedures for direct count plating. Samples were placed in enrichment broth for detection of Salmonella. Total plate counts were determined from pour plates of standard plate count agar (PCA). Fecal coliform and fecal streptococci were enumerated by pour plating on m-FC agar and m-Enterococcus agar, respectively. The m-FC agar was solidified with the addition of 7.5 g of agar per liter to m-FC broth. Three dilutions for each sample were used and triplicate plates were used for each dilution. Standard plate count and Enterococcus plates were incubated at 35°C for 48 hours. Fecal coliform plates were incubated at 35°C for four hours then twenty hours at 44.5°C. Colony counts were made after incubation and selected colonies were picked for biochemical identification.

One-milliliter samples of wastewater and two serial dilutions of each sample were incubated in Selenite cysteine broth for 24 hours at 35°C. Three tubes of broth were used per sample dilution. Additional samples of 10 and 100 ml per site were added to appropriate volumes of triple strength Selenite cysteine for the sampling periods May to August.

After 24 hours a loop of broth from each sample dilution was streaked on to a Brilliant Green agar plate and incubated at 44.5°C for 24 hours. sp. Salmonella-like colonies were then picked for further identification and typing.

FISH SAMPLES

On February 11, 1983, prior to stocking the silver carp into the wastewater ponds, the fish from the holding ponds were placed into concrete holding tanks and treated with 200 ppm formalin for one hour to control external parasites. Bacteriological samples from three representative lots of ten fish each were taken to determine bacterial levels prior to stocking.

After stocking, ten fish from each pond were sampled every 30 days. Attempts were made to isolate bacteria from the body surface mucous, kidney and intestine of these fish.

A sterile metal template was used to isolate 1 cm square area of mid-lateral body surface for mucous samples from each fish. This area was swabbed with a sterile cotton swab and then aseptically placed in a tube of phosphate-buffered saline. The mucous samples from the ten fish from each pond were pooled, agitated with a vortex mixer, and appropriate serial dilutions made. Standard plate count, fecal coliforms, and fecal streptococci were enumerated after the appropriate period. The media used, incubation temperatures, and periods were the same as for the water samples. Bacterial counts were expressed as per square centimeter.

After the mucous sample was taken, the body cavity of each fish was aseptically opened and the trunk kidney removed. The kidney samples of the ten fish from a single pond were pooled into one sterile glass tube, reweighed and 10 ml of phosphate-buffered saline added. After homogenizing with a Waring blender, the sample was serially diluted and plated following the earlier discussed procedures.

Approximately 3 cm of the posterior portion of the intestine of each fish were removed aseptically and placed into a weighed tube. Ten intestine samples of fish from the same pond were pooled into one tube, reweighed and 10 ml of phosphate-buffered saline added before homogenizing. Serial dilutions were made and plated following the earlier discussed procedures. The bacterial counts of both kidney and intestine samples are expressed on a per gram of tissue basis.

SPECIAL PROCEDURES

Colony Types

A study was conducted to trace the incidence of certain distinct colony types over time. Surface colonies (those growing on the agar surface) with distinct morphology and color were chosen from the total plate count agar plates. Six apparent types of bacteria were followed from the beginning of sampling program and two more types were added when they appeared later in the experiment (Table 1). These colony forming units were designated Bacteria Type (BT) I through BT VIII. The colony types from water samples were followed from February through August and from fish samples from March through August.

All triplicate PCA plates from each pond and fish samples had the total number of each distinct Bacteria Type and the total number of surface colonies counted and recorded for each plate to generate data from which ratios and percentages of bacterial populations could be derived.

Following this, five of each Bacteria Type, if present, were picked randomly from all pond samples to verify the composition of the type. Colonies picked were restreaked on PCA for further isolation and identification.

Random Samples

The months of January, April, and July were chosen as representative of winter, spring, and summer season conditions, respectively. During these months isolates were picked at random from countable plates of PCA. The January water sample consisted of 30 picked isolates, ten from each pond. The April and July water samples consisted of 45 isolates, 15 from each pond, 5 from each location.

Bacterial colonies from the fish samples were randomly picked during February and from April until August. The February sample is representative of fish prior to stocking into the wastewater ponds. February's sample consisted of 15 colonies from the kidney samples, 5 from each pond group. Six colonies were picked randomly from the mucous sample of the three lots of fish, and ten colonies from the intestines. Random samples from April through August consisted of 15 colonies picked from the kidney, intestine, and mucous samples respectively, 5 from each pond group.

To randomize the sampling a numbered grid made from acetate was placed under the petri plate. Numbers from one to forty-six mark off each zone on the grid. Grid zone numbers were picked randomly and any colonies that lie nearest the upper right corner of the grid zone were picked and restreaked onto plate count agar and incubated at 35 degrees C for 24 hours to isolate pure colonies for identification. This process was repeated using any of the appropriate three replicate plates until the designated number of colonies were picked and restreaked.

Characteristic red colonies were picked from the water and fish sample plates of m-Enterococcus agar randomly as described above to verify counts as Streptococcus and to determine predominant species isolated. Five

characteristics red colonies were picked from January's water sample and each month starting with February until August, 25 red colonies were picked for count verification and identification. Twelve were from the water and thirteen were from the fish.

Bacterial Identification

For all bacteria isolated, identification procedures involved the following classical biochemical tests: Gram reaction and morphology, oxidase reaction, catalase reaction, reactions on Triple Sugar Iron Agar, reactions in Glucose Motility deeps, growth on MacConkey's Agar and motility. These tests were used to place unknowns into groupings and to aid in properly choosing the appropriate Minitek system (BBL) and additional biochemical tests for presumptive identification.

SALMONELLA EXPOSURE STUDY

Silver carp were exposed to Salmonella typhimurium for twenty-nine days then were examined to determine if they were carriers of Salmonella. These fish were then transferred to freshwater not containing Salmonella and sampled after 5 and 15 days to determine if the fish were still carriers.

A stock culture of Salmonella typhimurium was developed to be resistant to 1 mg per ml of streptomycin sulfate. This streptomycin resistant Salmonella (S.R. Salmonella) was used to inoculate 7.3 m^2 static water pools. These pools had an average depth of 0.46 m. Inoculum of 1000 to 100,000 cells/1 were tested to determine the 24 hour die-off rate. The die-off rate was determined from grab samples taken at 0, 1, 2, 4, 8, 16, and 24 hours after inoculation. Three 100 ml samples were taken from each of five randomly selected pools each time period. The grab samples were taken 30 cm below the water surface using

sterile jars. The samples were then taken to the laboratory and aliquots of each sample were filtered through a 0.45 um porosity membrane filter by vacuum. Aliquot size varied from 1 ml at 0 hrs to 100 mls at 24 hrs. Control samples of 100 mls from uninoculated pools were also filtered. All samples were held on ice and filtered within six hours. Each filter was placed on Salmonella Shigella agar plates that contained 1 mg of streptomycin per ml. The plates were then incubated at 37°C for 24 hours and the colonies then counted.

It was found that an inoculation rate of 10,000 cells per liter of pool volume would give a mean concentration of 18.7 cells Salmonella per 100 ml after a 24 hour period. Lots of inoculum were prepared in advance and frozen in 50 ml volumes and held a minimum of 24 hours before use. Three subsamples of each lot were tested to determine the number of viable cells per ml using micro drop technique on SS agar containing 1 mg of streptomycin per ml.

Ten pools were inoculated daily with the streptomycin resistant Salmonella over a 40 day period. On three occasions during this time period, five pools were picked at random and the die-off rate of Salmonella was determined.

On the eleventh day the Salmonella containing pools were stocked with silver carp at 14 fish per pool. These fish had previously been examined and found to be free of Salmonella. The fish were held in the pools that continued to receive a daily inoculum of 10,000 Salmonella/1 for 20 days. At the end of that period, the fish were divided among six pools containing freshwater free of Salmonella. After five days another sample of fish from each pool was examined. This was repeated at fifteen days after transfer also.

The mucous, kidney and intestine of the fish in each sample were examined. Each sample contained five fish. The fish were anesthetized with quinaldine

and taken to the laboratory for examination. The mucous was sampled by swabbing the length of each fish ten times along the laterial line area using a sterile cotton swab premoistened in sterile phosphate buffered saline. The cotton tips were then cut aseptically into tubes containing 10 ml of double strength selenite-cysteine broth. Each tube was then incubated at 37°C for 24 hours then a loop of broth streaked on SS/strep plates. The plates were incubated 24 hours at 37°C and counted after that period.

The pools used in the 40-day study were monitored daily in the morning for dissolved oxygen, temperature, pH, and secchi disc visibility. These parameters were also measured weekly at 17:00 to 18:00 hours. Attempts were made to maintain a plankton density that would give a secchi disc reading of 30 cm or less by the additions of liquid diammonium phosphate fertilizer.

ANTIBODY EVALUATION

Silver carp from the three wastewater ponds and one pond not receiving wastewater were examined for the presence of agglutination antibodies. After that time ten fish were bled from each pond and the blood pooled to give one sample per pond. The sera were collected and heat inactivated. They were then used to determine by agglutination the presence of antibodies against selected bacteria. Serum dilutions of 1:10, 1:20 and 1:40 were mixed with a No. 4 McFarland suspension of the selected bacteria in microtiter plates. Controls of sera and saline, and saline and bacteria were prepared. The microtiter plates were sealed and incubated 24 hours at 35°C, then agglutinations recorded.

The bacteria used were isolates obtained from the water of the wastewater ponds, from silver carp cultured in those ponds and one stock culture. The isolates used were as follows:

1. Enterobacter cloacae, from fish mucous
2. Escherichia coli, stock culture
3. Bacillus pumilus, from pond water
4. Pseudomonas pseudoalkaligenes, from fish kidney
5. Bacillus sp., from fish intestine
6. Plesiamonas shiegelloides, from fish intestine
7. Klebsiella pneumoniae, from fish intestine
8. Aeromonas hydrophila, from fish intestine
9. Klebsiella ozaenae, from fish intestine
10. Klebsiella ozaenae, from fish intestine
11. Klebsiella pneumoniae, from fish intestine
12. Klebsiella pneumoniae, from pond water
13. Escherichia coli, from fish intestine
14. Escherichia coli, from fish intestine
15. Edwardsiella tarda, from fish intestine
16. Enterobacter agglomerans, from fish intestine
17. Enterobacter cloacae, from pond water
18. Enterobacter cloacae, from pond water
19. Escherichia coli, from pond water
20. Escherichia coli, from pond water
21. Edwardsiella tarda, from pond water
22. Enterobacter sp., from pond water
23. Edwardsiella tarda, from fish intestine
24. Salmonella sp., from fish intestine

BACTERIAL FLORA OF FISH BEFORE AND AFTER PROCESSING

Hybrid bighead-silver carp were taken from a pond not receiving domestic wastewater and cultured one month in the wastewater ponds earlier described. After that time ten fish from the wastewater pond and ten fish from the original non-wastewater pond were examined for bacteria.

Each lot of fish was held separately on ice for less than three hours after being collected until being examined. Bacterial counts for total plate count, fecal coliform, fecal streptococcus, and Salmonella were determined from the following sites: pond water, fish body surface mucous, intestine, body surface mucous after processing, and flesh of processed fish. The water samples were taken 10 cm below the pond surface near the center of each pond. The body surface mucous of each fish was sampled for total plate count, fecal coliforms and fecal streptococci by swabing a 1 cm^2 area using a sterile cotton swab and template. Samples from the ten fish of each lot of fish were pooled and vortexed in sterile saline. Serial dilutions were then made and pour plated with the approximate media. A separate 10 cm^2 area was swabed on each fish then samples from the same lot of fish pooled in sterile saline. Serial dilutions were made and added to selenite cysteine broth.

The body cavity of each fish was entered aseptically and approximately 3 cm of the intestine nearest the anus were removed. This sample was pooled with similar samples of intestine in a preweighed sterile test tube. The weight of intestine was then determined and homogenized in sterile saline. Serial dilutions were then made and pourplated with appropriate media and inoculated into selenite cysteine broth. In addition to the attempts to isolate Salmonella from pooled intestine samples, the intestine of each individual fish was

examined for Salmonella. Another section of approximately 3 cm of lower intestine was removed from each fish and homogenized in sterile saline and inoculated into selenite cysteine broth.

Each fish was then scaled and eviscerated and rinsed in chlorinated tap water. The body surface mucous was sampled as earlier described for total plate count, fecal coliform, fecal streptococci and Salmonella.

A section of flesh approximately 3.5 g was removed with a sterile knife from an area anterior to the dorsal fin of each fish. Each section contained skin and dorsal musculature. The ten sections from each lot of fish were then homogenized in sterile saline. Again serial dilutions were made and plated with the appropriate media.

Total plate counts were determined after a 48 hour incubation at 35°C. Fecal streptococci were counted after 24 hours at 35°C. Fecal coliform plates were incubated 4 hours at 35°C and 20 hours at 44.5°C. Selenite cysteine broths were incubated at 35°C for 24 hours then plated on Brilliant Green agar plates. These plates were incubated for 18 hours at 44.5°C then examined. Salmonella-like colonies were restreaked on TSA for further identification. Isolates were tentatively confirmed by their reaction to TSI, Christensen's urea and lysine decarboxylase.

Bacterial counts from the body surface are expressed as number per cm^2 while counts from the intestine and flesh are expressed as number per gram of tissue. Salmonella are expressed as most probable number per cm^2 or gram for the pooled samples. The individual intestine samples were recorded as presence or absence of Salmonella.

SECTION 5

RESULTS AND DISCUSSION

SILVER CARP PRODUCTION

Wastewater ponds were an excellent environment for the production of silver carp. The net production was an average of 1,309.2 kg/ha with a standing crop of 1,513.1 kg/ha. The average weight of the fish produced was 204.6 grams. The average survival was 84.1% when those removed during sampling are added.

The growth rate of silver carp is related to the water temperature (Table 1). As the water temperature began to increase the growth rate also did. Little growth was seen when the water temperature was below 15°C, a higher growth rate was seen from 15°C to 18.5°C and even a higher rate with temperatures above that.

The reduced growth rate in the latter months is an effect of carrying capacity of the system. If the culture period had continued it would have been anticipated that the growth rate would continue to be reduced and at one point stop.

Silver carp were surprisingly resistant to the wastewater environment and handling. No excessive mortality occurred as a result of the monthly sampling. Oxygen levels decreased to 1 mg/l on several occasions as the result of plankton dieoffs but had little apparent effect on silver carp survival.

BACTERIAL COUNTS FROM WASTEWATER (TABLE 2)

Influent

The total plate count from the influent water did not differ significantly over time. The values ranged from 2.44×10^5/ ml to 3.25×10^6/ ml over the eight-month period. The fecal coliform counts did vary significantly with time, ranging from 1.79×10^3/ ml to 3.36×10^4 /ml. There was a trend toward

Table 1.

Average Growth of Silver Carp

	Mean weight, g	Daily mean gain per fish per month, g	Mean monthly water temperature °C
March	20.70		13.16
		0.09	
April	23.30		14.89
		0.88	
May	49.58		18.47
		2.15	
June	114.17		23.50
		1.95	
July	172.58		26.57
		1.07	
August	204.59		28.00

reduced values in the warmer months. Such a trend would be expected and the warmer water would increase the efficiency of the wastewater treatment plant. Also the flow through the plant is reduced during that period of the year, thus a longer retention time in the plant helping its efficiency. While fecal coliform counts might be reduced, non-coliforms associated with the filter might increase thus little change in the total plate count. There were no significant differences in the streptococcus values over time. The values ranged from 3.64 x 10^2 to 1.83 x 10^3/ ml.

Midwater

There were no significant changes in the total plate count over time in the midwater samples. The values varied from 3.82 x 10^4/ ml to 3.27 x 10^5/ ml. The fecal coliform counts did vary significantly with time. Higher values occurred January to May with lower values in June to August. The values ranged from 7.35 x 10^3 in January to 7.57 x 10^2 in August. The fecal streptococcus values varied significantly by month but with no apparent trend. The greatest value occurred in April with lesser values for March and May. The lowest value of 6.58 x 10^1 occurred in May.

Effluent

Effluent values for total plate count did not differ over time nor were there any apparent trends in the counts. Values ranged from 4.24 x 10^4 to 2.49 x 10^5/ ml. There was a trend in fecal coliform values to become reduced over time but the values were not statistically different. The values ranged from 4.86 x 10^2 to 7.56 x 10^3/ ml. Monthly fecal streptococcus counts did differ significantly but there was no apparent trend over time. The values ranged from 3.67 x 10^1 in July to 2.65 x 10^2 in April.

Relationships Between Sites

There were significant reductions between influent and midwater values for total plate count, fecal coliform, and fecal streptococci (Appendix I). There was a 68.7% reduction in total plate count values from influent to midwater counts. There was a range in this reduction of 16.38 to 96.73% with no apparent trend over time. There was an 81.43% reduction in fecal coliform values between influent and midwater values. There was no trend over time and a range in percent reduction of 62.9 to 88.75%. There was a 71.40% reduction in fecal streptococcus values between influent and midwater. The percentages ranged from 94.5% to 45.24%. The reduction was notably less in the months of June to August.

The changes between midwater and effluent varied depending on the type of count. The mean reduction in total plate count was 18.87% between midwater and effluent. There was a wide range in monthly differences. In January there was a 26.2% increase between the two sites while in February there was a 76.58% decrease. There were no apparent trends over time. There was a 38.20% increase in fecal coliform counts, but again with a wide range in monthly values. The values ranged from a decrease of 43.95% in April to a 290% increase in July. The fecal streptococcus value decreased by 22.46%. There again was a wide range in percentages. In February there was a 79.76% increase while in July there was a 91.26% decrease. There was an apparent trend over time for fecal streptococci with the greatest increases from midwater to effluent occurred in January and February while the greatest decrease occurred in July and August.

The percent changes between influent and midwater, and midwater and effluent on occasions had no clear cut relationship. In February there was a

Table 2.

Bacterial Counts per ml from Water for All Ponds

	Influent	Midwater	Effluent
Total Plate Count			
1-12-83	5.21×10^5	1.45×10^5	1.83×10^5
2-11-83	3.25×10^6	1.32×10^5	1.19×10^5
3-14-83	1.00×10^6	1.81×10^5	4.24×10^4
4-13-83	2.44×10^5	1.17×10^5	8.58×10^4
5-16-83	5.62×10^5	3.27×10^5	2.49×10^5
6-13-83	3.01×10^5	2.52×10^5	2.08×10^5
7-13-83	1.46×10^6	1.11×10^5	6.79×10^4
8-12-83	1.17×10^6	3.82×10^4	4.44×10^4
Fecal Coliforms			
1-12-83	3.36×10^4	7.35×10^3	4.31×10^3
2-11-83	1.56×10^4	3.22×10^3	7.56×10^3
3-14-83	4.10×10^4	4.80×10^3	3.34×10^3
4-13-83	3.21×10^4	1.19×10^4	6.67×10^3
5-16-83	7.52×10^3	1.32×10^3	1.32×10^3
6-13-83	1.79×10^3	2.79×10^2	1.09×10^3
7-13-83	4.79×10^3	6.18×10^2	8.21×10^2
8-12-83	6.73×10^3	7.57×10^2	4.86×10^2
Fecal Streptococcus			
1-12-83	1.83×10^3	1.00×10^2	1.38×10^2
2-11-83	3.64×10^2	8.4×10^1	1.51×10^2
3-14-83	8.04×10^2	7.69×10^1	3.92×10^1
4-13-83	1.33×10^3	3.99×10^2	2.65×10^2
5-16-83	4.01×10^2	6.58×10^1	7.67×10^1
6-13-83	3.89×10^2	1.45×10^2	1.20×10^2
7-13-83	7.67×10^2	4.2×10^2	3.67×10^1
8-12-83	1.40×10^3	4.83×10^2	4.78×10^1

95.9% reduction in total plate count values between influent and midwater and a 10% reduction between midwater and effluent. In March there was an 81.50% decrease between influent and midwater and a 76.58% decrease between midwater and effluent. In January there was a 72.17% decrease between influent and midwater while a 26.2% increase from midwater to effluent.

There was an apparent relationship in the changes between sites for fecal streptococcus values. In January and February there were 94.5 and 76.92% decreases between influent and midwater while there were 38.00% and 79.76% increases between midwater and effluent values, respectively. In July and August the amount of decrease between influent and midwater was less (45.24 and 65.5%) and at the same time the differences between midwater and effluent had become decreases of 91.26 and 90.10%. This trend of changing efficiencies is not as apparent for the months in between.

There was an overall 75.00% reduction in total plate count values from influent to effluent, 77.90% for fecal coliform and 83.84% for fecal streptococci. In general the total plate count, fecal coliform and fecal streptococcus values were reduced the greatest in July and August. A greater abundance of high fecal coliform and fecal streptococcus values for all sites occurred in April in relation to the other months. This was not true for total plate counts.

BACTERIAL COUNTS FROM FISH (TABLE 3)

Mucous

There was a major increase in total plate count values comparing mucous counts from fish before they were stocked into the wastewater ponds to counts obtained after stocking. Values ranged from $1.33 \times 10^2/cm^2$ before stocking to a peak of $6.37 \times 10^4/cm^2$ in May. The initial counts obtained may have

been reduced as the fish had been treated prophylactically with 200 mg/l of formalin for one hour for parasite control. This treatment which was given 24 hours before the bacterial examination was made may have also reduced the bacterial count from the mucous. The total plate counts continued to increase while the fish were being cultured in the wastewater ponds until May. After that date the counts progressively decreased. A similar response occurred with the fecal coliform counts. There was an increase from before stocking to after with a continued rise in counts until May and after that sample values gradually decreased. The fecal coliforms increased from $6.67 \times 10^1/cm^2$ before stocking to a maximum of $6.35 \times 10^3/cm^2$ in May then decreased to $1.53 \times 10^2/cm^2$ in August.

This decrease from May to August may be in part due to the immune response system of silver carp becoming more active as the water began to warm. It is common for the immune response of warm water fish to be minimum in cold water but as the temperature increases the immune response does also. The effect of temperature was earlier discussed in relationship to the increase in growth rate during the latter months of the study.

Fecal streptococcus values were low from the mucous. They were the greatest before stocking ($3.33 \times 10^1/cm^2$) and decreased to $0/cm^2$ for three months of the sampling period. Although there was variation in counts, there was no apparent trend in the fecal streptococcus values over time.

Kidney

There were significant changes in total plate count values from the kidney. The fish examined before stocking had the greatest level of bacteria ($1.62 \times 10^5/g$) with March and April samples also having high levels. May through

August had significantly lower levels than earlier months. There was a trend toward progressively lower counts through May with a progressive increase from June to August. Fecal coliform values ranged from 2.22×10^1 to 9.92×10^1/g. There were no significant differences over time. No fecal streptococci were ever isolated from the kidney at any sampling period.

Intestine

There was almost a two magnitude increase in total plate count from before the fish were stocked into the ponds to the first month of stocking. The maximum plate count values from the intestine occurred in May (2.62×10^8/g). This value was significantly greater than those of other months. Fecal coliform values also increased dramatically when the fish were stocked into the wastewater ponds going from 7.43×10^4/g to 4.45×10^7/g in March, which was the highest value for the sampling period. There were no trends in fecal coliform counts over time while the fish were in the wastewater ponds. There were also increases in fecal streptococcus counts after the fish were stocked into the ponds. There was a trend toward higher fecal streptococcus values in the later months of the study. March and April values were lower than those from May through August.

The large increase in total plate count, fecal coliform and fecal streptococcus values after stocking is due in part to the lack of fecal material in the intestine of the fish examined before stocking. These fish were held in clean water several days before stocking and had defecated much of the fecal material before the fish were examined. Samples taken after fish were stocked into the ponds, were of fish which had recently been feeding thus with a full intestinal tract and more feces.

Table 3.

Bacterial Counts by Location

Fish

	Total Plate	F. Coliform	F. Streptococci
		Mucous/cm^2	
2-11-83	1.33×10^2	4.00×10^1	3.31×10^1
3-14-83	8.00×10^3	5.67×10^2	0.0
4-13-83	1.99×10^4	6.00×10^2	2.22
5-16-83	6.37×10^4	6.35×10^3	0.0
6-13-83	1.61×10^4	2.92×10^3	8.8
7-13-83	1.05×10^4	4.33×10^2	0.0
8-12-83	1.77×10^3	9.20×10^1	1.1
		Kidney/g	
2-11-83	1.62×10^5	1.39×10^2	0.0
3-14-83	3.00×10^4	4.4×10^1	0.0
4-13-83	2.93×10^4	1.04×10^2	0.0
5-16-83	1.41×10^3	2.22×10^1	0.0
6-13-83	5.09×10^3	1.87×10^1	0.0
7-13-83	7.22×10^3	1.65×10^2	0.0
8-12-83	1.09×10^4	1.24×10^1	0.0
		Intestine/g	
2-11-83	2.05×10^6	7.43×10^4	4.40×10^1
3-14-83	1.01×10^8	4.45×10^7	1.86×10^4
4-13-83	4.90×10^7	1.04×10^7	3.97×10^4
5-16-83	2.62×10^8	2.63×10^7	6.00×10^5
6-13-83	5.87×10^7	1.92×10^7	8.10×10^5
7-13-83	6.32×10^7	2.31×10^7	8.22×10^4
8-12-83	3.67×10^7	2.62×10^6	3.16×10^5

Relationship Between Sites and Types of Counts

Fecal coliform values from the mucous averaged 89.08% less than the total plate count with the least difference being 69.99% less from the samples taken just before stocking. Fecal streptococcus values were 99.97% less than the total plate count values from the mucous with the least difference also occurring at the time of stocking. Fecal coliform values were 99.4% less than the total plate count values from the kidney. The fecal coliform values from the intestine were 82.01% less than those of the total plate count while the fecal streptococcis were 99.63% less.

The ratio of fecal streptococcus to fecal coliform and total plate count in the intestine, after the fish had been in the wastewater ponds, decreased from the initial ratio found before stocking. These ratios increased in the mucous for the same period. The ratios of fecal streptococcus to fecal coliform and total plate count from the intestine were less than those same ratios from either influent, midwater, or effluent. The ratio of fecal coliform to total plate count in the intestine was less than the ratio between those counts for the water samples. Thus fecal coliform and fecal streptococci were a relatively greater percent of the bacterial flora of the intestine in relation to the total count than they were in the water.

The difference in the ratios of fecal streptococcus to fecal coliform and total plate count, from the mucous, were greater than those ratios from the water samples. The differences in fecal streptococcus to fecal coliform and total plate count were greater ratios in the mucous samples than from the intestine. Thus fecal streptococci were a relatively smaller percentage of the bacterial flora from the mucous than they were in the water or fish intestine.

The ratio of fecal coliforms to total plate count was less in the mucous samples than that from the water. The ratios of fecal coliforms to total plate count were similar for the mucous and intestine. Fecal coliforms were therefore a greater percentage of the flora from both the mucous and intestine than they were from the water.

There appeared to be some direct relationship between total plate count from water and fish when the means for the three ponds are considered. The largest total plate count values for the midwater and effluent samples occurred in May. This was paralleled with the largest total plate counts from mucous and intestine occurring in that month. There is also a larger fecal coliform count from the mucous in that month. But at the same time, these values increased, the total plate count from the kidney had decreased to the lowest level found during the sampling period. The peaks of fecal coliform and fecal streptococcus values from the midwater in April were not matched by peaks of fecal coliform or fecal streptococcus values from the mucous or intestine at that time. When the relationships between counts for the various sites for an individual pond are considered these relationships are less clear. High values for the above mentioned sites did occur in April but the highest value for that site did not necessarily occur in that month.

The levels of total plate count, fecal coliform and fecal streptococci in influent waters appeared to have little relationship to those found from the fish. The increases or decreases in fecal streptococci from the intestine or mucous did not appear to have any direct relationship to those changes occurring in the water samples.

BACTERIAL COLONY TYPES (APPENDIX II and III)

Seven bacterial colony types were followed during the study. Representative colonies of each type were picked monthly from the total plate counts from water and fish samples for identification.

Type I occurred throughout the study period. Forty-five of the forty-nine representative colonies of Type I were Flavobacterium. Type II was composed of two genera. Pseudomonas was the dominant genera through May. Microcyclus became dominant in those identified from June to August. Type III was made up of several similar appearing forms. Through May it was represented by several different enterics. After May the enterics disappeared and a similar appearing colony, which was a Bacillus was found. Type IV was only present in the early months and disappeared with time. Twenty colonies were identified for this type; fourteen were Moraxella. Type V was predominatly Klebsiella. Forty-six of the sixty colonies picked representing this type were Klebsiella, seven were Enterobacter, two E. coli and one Citrobacter and one Acinetobacter. There was no change in the composition of Type V in regard to time. There was a change over time in the composition of the fifty-five colonies picked to represent Type VI. Up through May, eighteen of the colonies identified were Enterobacter and seven Aeromonas of the twenty-five picked. After May no Enterobacter were found and only Aeromonas was present. Type VII did not become apparent until July. It was composed solely by Bacillus.

The occurrence of these seven types of bacteria were noted by the site where they were found and expressed as a percentage of the total bacterial count from the surface of the total plate count plates for the given site. Type I was generally a low percentage of the population from the influent samples except in

August when it increased to 20.16%. Its relative abundance was greater in the midwater and effluent than in the influent. In the midwater it became 33.36% of the population in May while the overall average was 12.02%. This peak in May also corresponds with the peak in total plate count values from the midwater which also occurred in May. Type I was very rare from the intestine being only 0.045%. The mean for Type I in the mucous samples was 3.02%. It is thus slightly more common in the mucous than the intestine but less common from fish than the midwater and effluent.

Type II was 0.26% of the surface total plate count population of the influent and increased to 7.75 and 6.78% of the midwater and effluent populations. There were considerable variations in its abundance both in the midwater and effluent. This was particularly true in pond B where the midwater and effluent values respectively rose to 25.75% and 30.61% in May and in July to 78.49% and 55.25%. The relative occurrence of Type II from the intestine and mucous were only 0.03% and 3.86% respectively.

Type III occurred in such a low percentage at all sites and was a mixture of organisms that no discussion of this type will be given.

Type IV was only observed from February to April and completely disappeared after that date. During that time it averaged 6.74% of the influent population, 2.0% of the midwater and 6.08% of the effluent. It comprised 1.19% of the flora from the mucous and 5.24% from the intestine in March but was not found during any other month.

Type V was the dominant type in the influent, midwater and effluent sites of the seven types followed. In the influent water it was 21.82% of the surface total plate count, 11.10% of midwater counts and 10.36% of effluent counts. It

was 9.76% of the intestinal population and 7.95% of the mucous. There were considerable variations at each site over time but no trend was evident. There was a decrease in the percentage when influent is compared to midwater and effluent percentages. Peak percentages in the intestine or mucous did not appear to correspond directly with peak percentages from the midwater or effluent. It thus would appear that even though Type V (Klebsiella) was abundant at all sites there appeared to be little interaction between populations in water and fish in regard to population changes.

Type VI was made up of Enterobacter and Aeromonas. It was predominately Enterobacter through April. In May it was 40% Enterobacter and 60% Aeromonas. After May it was 100% Aeromonas. The percentage of Type VI was 8.25% in the influent, 8.88% for the midwater and 8.56% in the effluent. There was a range in these values of 0 to 25% but there was no apparent trend at any site over time. The percentage of Type VI in the intestine was 41.37% with maximum peaks occurring in May of 82.11% and in July of 61.61%. Type VI was 30.60% of the surface plate count values for the mucous samples. Peak percentages in mucous samples occurred in May of 56.24% and in August of 46.40%. The peak in May corresponds with the period when the components of Type VI had shifted from Enterobacter to a mixture of Enterobacter and Aeromonas. The later peaks were represented by Aeromonas. Type VI was the dominant colony type from the intestine and mucous of the seven colony types followed. It occurred at a much greater percentage in the mucous and intestine than in the water. No other colony type that was studied occurred at a greater percentage from fish than from water. There was no apparent relationship between the peak occurence of Type VI in the intestine and mucous to the peaks found in the water.

Type VII became noticeable only in the last two sampling periods. In July and August this Bacillus was 1.77% of the influent population, 5.87% of the midwater, and 4.55% of the effluent. In the intestine it was 0.67% and 1.96% in the mucous. It might be noted that this form of Bacillus was evident only in July and August. Similarly Type III had shifted from enterics to being a Bacillus in the June to August samples.

In summary, the seven colony types followed in this study were found from both the water and fish but their relative percentage of the surface plate count varied depending on the site. There appeared to be little correlation for fluctuations of most types between relative percentages from water or fish.

IDENTIFICATION OF COLONIES PICKED AT RANDOM

Total Count

Randomly selected colonies were selected for identification from the total plate count plates for January, April, and July water samples. Colonies were randomly selected from fish samples in February, and April through August. the species found are given in Tables 4 and 5. There was an increase in the gram negative non-fermenters over time from the water samples while the enterics, excluding Aeromonas, decreased. Gram positive bacteria were more common in the July water samples than those earlier.

Gram negative non-fermenters were much more common from the mucous than the intestine. The enterics were more common from the intestine than the mucous overall, but in the later sample it was more evenly divided. Isolates from the kidney were predominately Pseudomonas pseudoalkaligenes through May. In July there were predominately Aeromonas and in August were a mixture of the two.

Fecal Coliforms

Randomly selected colonies from the fecal coliform samples of April to August were identified. Eleven species were identified from the water. Ten species were identified from the fish. These ten species were also found in the water. The species found are given in Tables 6 and 7. Klebsiella pneumonae was the most frequently found species from the water and was more common in the effluent than influent or midwater. Klebsiella ozaenae, Enterobacter cloacae, and Escherichia coli were the next most common from the water.

Aeromonas hydrophila was the most common bacteria at all sites from fish. Several species were found from the intestine but not the mucous. Citrobacter amalomaticus was not found from the fish but did occur in low levels from the midwater in April and May.

Fecal Streptococci

Colonies were picked at random for identification from the monthly samples of water, fish intestine, and mucous plated on Enterococcus agar (Appendix V). No colonies were found from kidney samples plated on Enterococcus agar. There were no apparent trends in the species composition over time. Streptococcus faecalis comprised 60.7% of the isolates identified from the water and 47.3% of those from fish intestine. S. faecium was the second most abundant species both from the water and intestine. Other species found were S. durans, S. equinus. S. bovis, S. mitis, and S. lactis. Few streptococci were found from the mucous samples. The species found were S. faecalis, S. faecium, and S. bovis.

SALMONELLA FROM WATER AND FISH FROM WASTEWATER PONDS

Attempts were made to isolate Salmonella from fish and water each month as a part of the monthly sampling program. Salmonella were isolated from the water

Table 4.

Random Sample of Bacteria from Total Plate Count Samples of Water

Date of Sampling	1-83	4-83	7-83
Total Number Sampled	30	45	45
Number of Isolates			
Micrococcus			1
Bacillus firmus			1
Bacillus	1		3
Kurthia			2
Corynebacterium	1		1
Total Gram Positives	2		8
Flavobacterium		1	3
Acintebacter	10	4	15
Alcaligenes	1	1	
Bordetella		1	
Moraxella	1	3	1
Xanthomonas			1
Pseudomonas sp.	1	6	7
Total Gram negative Non-fermenter	13	16	27
Aeromonas	4	4	5
Plesiomonas		1	2
Microcydus			1
Enterobacter cloacae		6	1
Enterobacter agglomerans	1	5	
Enterobacter	2	2	
Citrobacter	2	1	
Klebsiella pneumoniae	2	7	2
Klebsiella oxytoca	3		
Salmonella			
Total Enterics	11	26	11

Table 5

Bacteria Identified from Random Samples of Fish

Date of Sampling	2-83			4-83			5-83		
Total Number Sampled	11	17	7	16	15	27	15	13	15
	int	kid	muc	int	kid	muc	int	kid	muc
Micrococcus	1		6			3			
Bacillus stearothermophilus									
Bacillus firmus									
Bacillus sp				1		1			2
Kurthia									
Corynebacterium									
Streptomycis									
Gram Positives	1		6	1		4			2
Flavobacterium									1
Acintebacter						3			
Alcaligenes						2			
Moraxella						1			1
Pseudomonas sp.						1			
Gram negative Non-fermenter						7			2
Pseudomonas pseudoalkaligenes		15			15			10	
Aeromonas sp	9			3		1	7		4
Plesiomonas sp									
Enterobacter cloacae				7		2	7	3	5
Enterobacter aggl				3					
Enterobacter sp.			1			2			
Eshcherichia coli									
Citrobacter							1		
Klebsiella pneumonia									
Klebsiella oxytoca				1					
Total Enterics	9	15	1	14	15	5	15	13	9

Table 5A

Bacteria Identified from Random Samples of Fish

Date of Sampling	6-83			7-83			8-83		
Total Number Sampled	16	15	20	15	18	21	25	15	18
	int	kid	muc	int	kid	muc	int	kid	muc
Micrococcus					1	1			
Bacillus stearothermophilus					2		1		2
Bacillus firmus							1		
Bacillus sp				1		1			
Kurthia						1	1		
Corynebacterium							3		
Streptomycis									1
Gram Positives				1	3	3	6		3
Flavobacterium	1								
Acintebacter			4			1			
Alcaligenes						1	1		
Moraxella			1						
Pseudomonas sp.									1
Gram negative Non-fermenter	1		5			2	1		1
Pseudomonas pseudoalkaligenes					8			15	
Aeromonas sp	8	15	8	12	4	9	8		8
Plesiomonas sp				1		1			
Enterobacter cloacae	2		2						8
Enterobacter aggl									
Enterobacter sp.	1								
Eshcherichia coli	1								
Citrobacter						1			
Klebsiella pneumonia							1		
Klebsiella oxytoca	2								
Total Enterics	14	15	10	13	12	11	9	15	16

Table 6.

Fecal Coliforms Identified from Water Samples

Date of Sampling	4-83			5-83			6-83			7-83			8-83		
Total Number Sampled	14	13	15	12	9	9	9	9	12	8	7	9	8	8	18
Percentage by species	inf	mid	eff	inf	mid	eff	inf	mid	eff	inf	mid	eff	inf	mid	eff
Klebsiella pneumoniae	29	38	60	8	0	56	22	33	42	25	29	44	25	25	50
Klebsiella ozaenae	36	31	7	0	11	11	22	0	0	13	0	11	13	25	6
Klebsiella oxytoca	7	0	0	0	0	0	0	0	0	0	0	11	0	0	0
Escherichia coli	21	8	27	0	0	0	33	11	17	13	29	0	25	13	22
Citrobacter freundii	0	0	0	17	11	0	11	22	0	13	0	0	0	0	0
Citrobacter amalonaticus	0	8	0	0	11	0	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	7	8	7	50	44	22	0	11	8	37	14	0	25	37	22
Enterobacter agglomerans	0	8	0	8	0	0	11	11	8	0	0	0	13	0	0
Enterobacter aerogenes	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0
Edwardsiella tarda	0	0	0	0	0	0	0	11	0	0	14	0	0	0	0
Aeromonas hydrophila	0	0	0	17	22	11	0	0	17	0	14	22	0	0	0

Table 7.

Fecal Coliforms Identified from Fish

Date of Sampling	4-83			5-83			6-83			7-83			8-83		
Total Number Sampled	14	0	0	15	7	8	13	8	9	16	9	3	12	8	7
Percentage by species	int	kid	muc	int	kid	muc	int	kid	muc	int	kid	muc	int	kid	muc
Klebsiella pneumoniae	20	0	0	47	0	0	0	0	0	19	0	33	8	13	0
Klebsiella ozaenae	7	0	0	0	0	0	0	0	0	6	0	0	17	0	0
Klebsiella oxytoca	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0
Escherichia coli	7	0	0	13	0	0	8	0	0	6	0	0	8	0	0
Citrobacter freundii	7	0	0	0	0	0	54	0	22	0	0	0	0	0	0
Citrobacter amalonaticus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterobacter cloacae	50	0	0	20	71	50	8	0	0	0	0	0	0	0	0
Enterobacter agglomerans	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0
Enterobacter aerogenes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Edwardsiella tarda	0	0	0	0	0	0	0	0	0	6	0	0	8	0	0
Aeromonas hydrophila	0	0	0	7	24	50	23	100	66	62	100	66	58	87	100

samples only in July and August and from fish only in July. Additional samples were taken during June, July and August to isolate Salmonella from the water. Most Probable Number values ranged from < 0.3 to 25 cells/100 ml in the influent. Midwater and effluent values ranged from <0.3 to 0.9 cells/100 ml. Salmonella was found in the July sampling from the intestine. There it occurred at 5.5 cells/g in pond B and 2.6 cells/g in pond C but was not found from pond A.

The Salmonella isolated were from nine serological groups of Salmonella enteritidis (Table 8 and Appendix IV). Serological group C-1 was the most common followed by B and C-2. The influent water had a greater number of serological groups than midwater or effluent.

BACTERIAL COUNTS FROM FISH HELD IN PONDS NOT RECEIVING WASTEWATER

Fish held in ponds not receiving wastewater were examined in April, June, and August. Total plate, fecal coliform, and fecal streptococcus counts were determined from the mucous, kidney, and intestine of the fish sampled (Table 9). The total plate count from mucous averaged $6.94 \times 10^1/cm^2$ and was considerably less than the mean count of $3.84 \times 10^4/cm^2$ from the fish reared in wastewater. The fecal coliform and fecal streptococcus counts from the mucous of non-wastewater fish were also less than that from the mucous of wastewater fish. The total plate count from the kidney was greater from non-wastewater fish while no fecal coliforms or fecal streptococci were found. The mean value for the total plate count from the intestine of $2.43 \times 10^7/g$ is similar to that of the fish from wastewater while the fecal coliform count was one magnitude less in the intestine of non-wastewater fish. Fecal streptococci were at $4.75 \times 10^2/g$ in the intestine of the non-wastewater fish compared to $3.11 \times 10^5/g$ from wastewater fish.

Table 8.

Number of Occcurrences of the Serological Groups of *Salmonella* Isolated

Serological Group	Influent	Midwater	Effluent
B	4	2	2
B^1	1		1
C-1	8	4	5
C-2	1		
C^1-2	2	3	2
D-1		1	
E-1	1		
E-2	1	1	
H	2		

Table 9.

Bacterial Counts of Silver Carp from Non-Wastewater

Total Plate Count

Date	Mucous	Kidney	Intestine	Water
4-20-83	$9.9 \times 10^1/cm^2$	$2.92 \times 10^3/g$	$3.9 \times 10^7/g$	$2.0 \times 10^2/ml$
6-21-83	$9.9 \times 10^1/cm^2$	$2.99 \times 10^3/g$	$2.56 \times 10^7/g$	$3.77 \times 10^3/ml$
8-12-83	$1.02 \times 10^1/cm^2$	$8.35 \times 10^4/g$	$8.2 \times 10^6/g$	—

Fecal Coliform Count

4-20-83	0	0	$2.7 \times 10^5/g$	$1.13 \times 10^1/ml$
6-21-83	$2.13 \times 10^1/cm^2$	0	$4.0 \times 10^6/g$	$6.4 \times 10^2/ml$
8-12-83	$1.02 \times 10^1/cm^2$	0	$1.34 \times 10^6/g$	—

Fecal Streptococcus

4-20-83	0	0	$8.3 \times 10^2/g$	0
6-21-83	0	0	$1.03 \times 10^2/g$	0
8-12-83	0	0	$4.93 \times 10^2/g$	—

Colonies were picked for identification from the August samples of the non-wastewater fish. Six of the twenty colonies selected from the total plate count from mucous were Aeromonas and another six Actinobacillus-like organisms. Four Bacillus were isolated along with three gram positive cocci and one Corynebacterium-like organism. Three gram negative rods could not be identified and three colonies could not be reisolated in pure culture.

Gram positive bacteria were more common from the twenty-five colonies selected from the total plate count sample of intestine. Six Corynebacterium-like types were found along with four gram positive cocci, one Bacillus and one unknown gram positive rod. Four Aeromonas were isolated along with five other enterics and one unidentified gram negative bacterium. Aeromonas was the most common isolate from mucous and intestinal samples on fecal coliform agar. All of the ten isolates from the intestinal samples on Entercococcus agar were Corynebacterium-like organisms.

SIGNIFICANCE OF THE BACTERIAL LEVELS FROM FISH

Levels of bacteria found from fish in this study are similar to that of domestic animals or fish from natural waters (Table 10). A lower percentage of fish were found to have Salmonella in the intestine than typically would occur in swine being held for slaughter. Fecal coliforms, characteristic of homoiothermic animal feces, were found in the pond environment and occurred in the intestine of fish at levels similar to that from mammals. It would appear, as with other animals, the intestine of fish can be a site for the multiplication of bacteria. The presence of Aeromonas in the fecal coliform counts makes it more difficult to interpret the significance of the fecal coliform counts.

The significant point regarding the bacterial levels in the fish intestine is although the fish were cultured in an environment containing indicators of

bacterial pathogens, these indicators occurred at levels in the intestine similar to domestic animals. Current regulations will permit that domestic animals having these levels of bacteria in the intestine be processed for human consumption. Sanitary procedures are established that permits the processing of cattle and swine without excessive contamination even though indicators of bacterial pathogens may be found in the intestine at levels similar to that found from the fish in this study. Thus if bacterial contamination were the only factor determining if fish reared in wastewater could be utilized then such fish should be considered suitable for processing. Other animals are being processed with similar levels of bacteria. The key will be not the levels of bacteria present from fish before processing but, as with other meat products, the levels of bacteria occurring after processing.

RELATED STUDIES

Antibody Production by Silver Carp

Sera of fish from the three wastewater ponds and of fish from a pond not receiving wastewater were examined for the presence of agglutinating antibodies against twenty-three bacterial isolates listed in the experimental procedures for antibody production by silver carp.

Measurable antibody production was found against 7 of the 23 isolates tested. The species giving agglutination were: 2. Escherichia coli, stock culture; 3. Bacillus pumitus, pond water; 4. Pseudomonas pseudoalkaligenes, kidney; 5. Bacillus sp., intestine; 8. Aeromonas hydrophila, intestine; 11 and 12. Klebsiella pneumoniae. Sera of fish from non-wastewater gave agglutination against six bacterial isolates, nos. 2, 3, 4, 5, 8, 12. Sera from wastewater fish agglutinated wth isolates nos. 2, 3, 5, 6, 11, 12. Fish from

non-wastewater had titers against no. 4, while fish from wastewater did not. A very low titer was exibited toward isolate no. 11 by sera of wastewater fish but none by sera of non-wastewater fish.

An attempt to induce an immune response by orally inoculating viable Salmonella into the intestine of silver carp was made. In this laboratory study no agglutinating titer was evident from the sera at 35 days post-inoculation.

Effect of Transferring Fish from Wastewater to Freshwater

At the end of the wastewater study the fish were transferred by pond to 3 concrete holding tanks filled with non-wastewater. The fish were examined, at day 1, 3, 5, and 7 of confinement, for fecal streptococci and Salmonella.

No Salmonella was found from the wastewater fish before they were transferred to freshwater nor was there any found from the fish after being held in freshwater. Fecal streptococci were present in the intestine before being transferred to freshwater. Fecal streptococci continued to be evident in the intestine throughout the time the fish were in freshwater.

Effect of Exposing Fish to Salmonella

Silver carp were exposed to Salmonella for a twenty-nine day period. Daily inoculation of 10,000 cells/l were made, with daily die-off rates giving 18.67 cells/100 ml after 24 hours. After the twenty-nine-day exposure period, the fish were transferred to six pools of fresh water free of Salmonella. At that time fish were examined for Salmonella. Five out of six pools of fish had Salmonella present in the intestine at that time. It was also present from the mucous in one of the six groups. No Salmonella was found from the kidney.

After five days fish from each of the six pools were again examined. At that time no Salmonella was found from the mucous or kidney. It was present

from the intestine of fish from three of the five pools examined. At fifteen days after stocking Salmonella was not present in the mucous or kidney but was in the intestine of fish from three of five pools sampled. Fish from one pool were found to be negative for Salmonella when sampled at five days but the same pool had fish that were positive at fifteen days.

It would thus appear that when Salmonella are present at high levels in the water column, silver carp can harbor the organism in the intestine and to a lesser extent on the mucous. Transfer of carrier fish to fresh water will require a holding period of more than fifteen days to eliminate Salmonella from the intestine.

BACTERIAL FLORA OF FISH BEFORE AND AFTER PROCESSING

The bacterial counts are given in Table 11. There were no significant differences between wastewater and non-wastewater bacterial counts from the water samples or body surface mucous before processing. Total plate and fecal streptococci counts from the intestine were higher from the wastewater cultured fish than non-wastewater fish but fecal coliform counts were slightly higher in the non-wastewater fish. Levels of bacteria from samples of mucous and flesh were similar in processed fish from either group of fish. There was no significant change in bacterial counts from mucous samples before and after the fish were processed.

Salmonella was not found in the water, mucous or flesh samples. Salmonella was found in the intestine of fish from wastewater and non-wastewater. Salmonella concentrations from the pooled intestine samples were 32 cells (MPN)/gram of non-wastewater fish intestine and 1.03 cells/gram of wastewater fish intestine. Salmonella was detected from 4 of the 10 individual fish

intestine samples from non-wastewater and was not detected in the individual wastewater samples. The Salmonella isolated was identified as S. enteridis.

The bacterial counts from both groups of fish were similar to those of fish from wastewater discussed earlier in this study. Bacterial counts from body mucous and flesh of both groups of processed fish were generally less than similar counts reported for other processed meats, Table 12. It is significant to note that although Salmonella was detected in the intestine it is not detected from body mucous or flesh of processed fish.

The presence of Salmonella in the intestine of either group of fish may be due to an earlier exposure. The group of fish used in this study had been held in a pond fertilized with pig manure then transferred to an unfertilized pond and held three months before the start of this study.

Table 11.

Bacterial Flora of Fish Before and After Processing

	Water/ml		Mucous/cm^2		Intestine/g	
	Wastewater	Non-wastewater	Wastewater	Non-wastewater	Wastewater	Non-wastewater
Total plate count	1.67×10^3	$<10^3$	3.13×10^2	3.56×10^1	2.63×10^8	1.09×10^7
Fecal Coliform	8.6×10^1	3.10×10^1	6.22	<1	1.69×10^5	5.93×10^5
Fecal Streptococci	<1	<1	<1	<1	2.82×10^3	6.06×10^2

Dressed fish	Mucous/cm^2		Flesh/g	
	Wastewater	Nonwastewater	Wastewater	Nonwastewater
Total Plate Count	4.85×10^2	6.44×10^2	8.1×10	8.5×10^1
Fecal Coliform	<1	<1	<1	<1
Fecal Streptococci	<1	<1	<1	<1

Table 12.

Common Bacteria Levels from Processed Meats

	Beef [1]	Poultry [2]	Fish:Natural Waters [3]	Fish:Aquaculture [4]
Total Count /cm^2	10^5-10^7	10^3-10^6		
/g	10^6-10^7		10^4-10^5	
Fecal Coliform /cm^2				
/g				<3-10^3
Salmonella /cm^2	<3-18			

[1]Ayers, J. C., J. O Mundt, and W. E. Sandine, Microbiology of Foods, W. H. Freeman and Company, San Francisco

[2]Bryan, F. L. 1980. Poultry and Poultry Meat Products, In microbial Ecology of Foods, Vol. 2:Food Commodities, J. H. Silliken edit., Academic Press, New York.

[3]Shewan, J. M. 1961. The microbiology of seawater fish. In Fish as Food, Vol. 1, G. Borgstrom edit., Academic Press, New York.

[4]Andrews, W. H., C. R. Wilson, P. L. Poelma and A. Romero. 1977. Bacteriological survey of the channel catfish (Ictalurus punctatus) at the retail level. J. Food Science 42 (2):359-363.

Appendix I

Percentage Change in Bacterial
Counts Between Sites

Total Plate Count

	Influent to Midwater	Midwater to Effluent	Influent to Effluent
1-12-83	-72.17	+ 26.20	-64.88
2-11-83	-95.90	- 10.00	-96.34
3-14-83	-81.50	- 76.58	-95.76
4-13-83	-52.05	- 26.67	-64.83
5-16-83	-41.81	- 23.85	-55.70
6-13-83	-16.28	- 17.46	-30.90
7-13-83	-93.15	- 38.80	-95.34
8-12-83	-96.73	+ 16.23	-96.21
Mean	-68.70%	-18.87%	-75.00%

Fecal Coliform

1-12-83	-78.13	- 41.36	-87.17
2-11-83	-79.36	-134.78	-51.54
3-14-83	-88.29	- 30.42	-91.86
4-13-83	-62.90	- 43.95	-79.22
5-16-83	-82.45	- 0.0	-98.67
6-13-83	-84.41	-290.00	-39.11
7-13-83	-87.10	+ 32.55	-82.86
8-12-83	-88.75	- 35.79	-92.78
Mean	-81.43%	+ 38.20%	-77.90%

Fecal Streptococcus

1-12-83	-94.5	+ 38.00	-92.46
2-11-83	-76.71	+ 79.76	-58.52
3-14-83	-72.71	- 49.02	-95.12
4-13-83	-70.00	- 66.42	-80.08
5-16-83	-83.59	+ 16.57	-80.57
6-13-83	-62.73	- 17.24	-69.15
7-13-83	-45.24	+ 91.26	-95.22
8-12-83	-65.50	- 90.10	-99.29
Mean	-71.40%	- 22.46%	-83.84%

Appendix II

Abundance of Certain Bacterial Colony Types Expressed as a Percentage of Total Surface Colonies

POND A

Intestine

Colony	Total	I	II	III	IV	V	VI	VII	VIII
3-14-83	194	0.0	0.52	0.0	2.06	19.59	8.25		
4-13-83	103	0.0	0.0	0.97	0.0	7.77	26.21	0.0	0.0
5-16-83	288	0.0	0.0	0.0	0.0	5.56	95.49	0.0	0.0
6-15-83	32	0.0	0.0	0.0	0.0	21.88	28.13	0.0	0.0
7-14-83	105	0.0	0.0	0.0	0.0	5.71	38.10	0.0	0.0
8-12-83	130	0.77	0.0	0.0	0.0	3.08	45.38	1.54	0.0

Kidney

Colony	Total	I	II	III	IV	V	VI	VII	VIII
3-14-83	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
4-13-83	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
5-16-83	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
6-15-83	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
7-14-83	138	1.45	4.35	0.0	0.0	0.0	18.84	0.0	45.65
8-12-83	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Mucous

Colony	Total	I	II	III	IV	V	VI	VII	VIII
3-14-83	28	0.0	0.0	0.0	10.71	17.86	17.86		
4-13-83	37	2.70	0.0	0.0	0.0	10.81	5.41	0.0	0.0
5-16-83	196	2.55	0.0	0.0	0.0	5.1	82.14	0.0	0.0
6-15-83	91	3.30	0.0	0.0	0.0	3.30	23.08	0.0	0.0
7-14-83	28	3.57	0.0	0.0	0.0	0.0	32.14	0.0	0.0
8-12-83	97	0.0	0.0	0.0	0.0	3.09	49.48	3.09	0.0

Appendix II

Abudance of Certain Bacterial Colony Types Expressed as a Percentage of Total Surface Colonies

POND B

Intestine

Colony	Total	I	II	III	IV	V	VI	VII	VIII
3-14-83	393	0.0	0.0	1.02	1.53	22.65	14.25	0.0	0.0
4-13-83	284	0.0	0.0	0.0	0.0	10.56	14.44	0.0	0.0
5-16-83	215	0.0	0.0	0.0	0.0	11.63	77.67	0.0	0.0
6-15-83	168	0.0	0.0	0.0	0.0	3.57	32.74	0.0	0.0
7-14-83	141	0.0	0.0	0.0	0.0	4.96	75.18	0.71	0.0
8-12-83	203	0.0	0.0	0.49	0.0	0.49	38.92	0.49	0.0

Kidney

Colony	Total	I	II	III	IV	V	VI	VII	VIII
3-14-83	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
4-13-83	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
5-16-83	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
6-15-83	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
7-14-83	78	0.0	0.0	0.0	0.0	0.0	0.0	0.0	96.15
8-12-83	131	3.81	0.0	0.76	0.0	0.0	0.0	0.76	87.02

Mucous

Colony	Total	I	II	III	IV	V	VI	VII	VIII
3-14-83	20	0.0	0.0	0.0	5.0	10.0	10.0		
4-13-83	79	5.06	5.06	0.0	0.0	18.99	10.13	0.0	0.0
5-16-83	97	8.25	0.0	1.03	0.0	9.28	18.56	0.0	0.0
6-15-83	40	5.00	0.0	0.0	0.0	2.5	37.5	0.0	0.0
7-14-83	43	11.63	0.0	0.0	0.0	2.33	32.56	2.33	0.0
8-12-83	45	0.0	0.0	0.0	0.0	6.67	40.0	0.0	0.0

Appendix II

Abundance of Certain Bacterial Colony Types Expressed as a Percentage of Total Surface Colonies

POND C

Intestine

Colony	Total	I	II	III	IV	V	VI	VII	VIII
3-14-83	73	0.0	0.0	0.0	0.0	10.96	13.70		
4-13-83	169	0.0	0.0	0.59	0.0	7.7	17.16	0.0	0.0
5-16-83	246	0.0	0.0	0.0	0.0	12.2	73.17	0.0	0.0
6-15-83	24	0.0	0.0	0.0	0.0	20.83	29.17	0.0	0.0
7-14-83	218	0.0	0.0	0.0	0.0	1.38	71.56	0.45	0.0
8-12-83	239	0.0	0.0	0.0	0.0	5.02	45.19	0.84	0.0

Kidney

Colony	Total	I	II	III	IV	V	VI	VII	VIII
3-14-83	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
4-13-83	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5-16-83	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
6-15-83	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
7-14-83	53	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.00
8-12-83	171	0.0	0.0	0.0	0.0	0.0	0.0	1.17	98.25

Mucous

Colony	Total	I	II	III	IV	V	VI	VII	VIII
3-14-83	13	7.69	23.08	0.0	0.0	15.39	7.69		
4-13-83	18	0.0	5.56	0.0	0.0	16.67	11.11	0.0	0.0
5-16-83	222	0.0	0.0	0.0	0.0	1.35	68.02	0.0	0.0
6-15-83	12	0.0	0.0	0.0	0.0	16.67	16.67	0.0	0.0
7-14-83	31	0.0	0.0	0.0	0.0	0.0	38.71	3.23	0.0
8-12-83	97	0.0	0.0	0.0	0.0	3.09	49.48	3.10	0.0

Appendix II

Abundance of Certain Bacterial Colony Types Expressed as a Percentage of Total Surface Colonies

POND A

Influent Water

Colony	Total	I	II	III	IV	V	VI	VII	VIII
2-12-83	40	2.5	0.0	10.0	12.5	10.0	0.0	*	*
3-14-83	172	1.16	0.0	1.74	5.81	15.12	9.3	*	*
4-13-83	160	5.0	0.63	0.63	2.5	16.88	18.13	0.0	0.0
5-16-83	162	5.56	0.0	1.23	0.0	10.49	9.26	0.0	0.0
6-15-83	119	0.84	0.0	0.0	0.0	26.89	7.56	0.0	0.0
7-14-83	314	6.37	0.0	0.32	0.0	43.31	2.87	0.0	0.0
8-12-83	390	20.77	0.0	0.0	0.0	14.36	15.90	15.90	0.0

Midwater

Colony	Total	I	II	III	IV	V	VI	VII	VIII
2-12-83	13	7.69	15.39	0.0	0.0	0.0	0.0	*	*
3-14-83	32	9.38	0.0	3.13	9.38	28.13	12.50	*	*
4-13-83	223	4.04	0.45	0.45	4.93	18.83	11.66	0.0	0.0
5-16-83	19	21.05	0.0	0.0	0.0	36.84	21.05	0.0	0.0
6-15-83	203	3.94	0.0	1.97	0.0	16.26	14.78	0.0	0.0
7-14-83	395	3.29	0.0	0.76	0.0	15.95	7.85	10.38	0.0
8-12-83	498	8.23	0.0	0.0	0.0	3.61	9.44	3.01	0.0

Effluent Water

Colony	Total	I	II	III	IV	V	VI	VII	VIII
2-12-83	63	12.70	1.59	4.76	12.70	4.76	0.0	*	*
3-14-83	22	22.72	0.0	0.0	4.55	9.09	4.55	*	*
4-13-83	254	3.94	0.79	0.79	0.39	17.32	0.79	0.0	0.0
5-16-83	36	11.11	2.78	0.0	0.0	19.44	8.33	0.0	0.0
6-15-83	133	6.02	0.0	2.26	0.0	16.54	12.78	0.0	0.0
7-14-83	96	5.21	1.04	2.08	0.0	14.58	19.79	4.17	0.0
8-12-83	257	5.06	0.0	0.0	0.0	7.39	10.51	5.48	0.0

Appendix II

Abundance of Certain Bacterial Colony Types Expressed as a Percentage of Total Surface Colonies

POND B

Influent Water

Colony	Total	I	II	III	IV	V	VI	VII	VIII
2-12-83	56	3.57	0.0	5.36	5.36	3.57	0.0	*	*
3-14-83	167	2.99	0.0	1.20	8.98	16.17	6.59	*	*
4-13-83	73	1.37	0.0	2.74	2.74	34.25	17.81	0.0	0.0
5-16-83	173	3.47	0.0	0.0	0.0	18.50	7.52	0.0	0.0
6-15-83	111	3.60	0.0	0.0	0.0	32.43	9.01	0.0	0.0
7-14-83	114	4.39	0.0	0.0	0.0	25.44	7.90	0.0	0.0
8-12-83	430	39.77	0.0	0.0	0.0	18.37	4.88	0.0	0.0

Midwater

Colony	Total	I	II	III	IV	V	VI	VII	VIII
2-12-83	2	0.0	0.0	0.0	0.0	50.0	0.0	0.0	0.0
3-14-83	13	0.0	0.0	0.0	0.0	15.39	7.69	*	*
4-13-83	148	1.35	0.67	1.35	0.0	27.03	15.54	0.0	0.0
5-16-83	435	54.02	25.75	0.0	0.0	3.45	1.15	0.0	0.0
6-15-83	184	3.80	5.43	2.17	0.0	11.96	10.87	0.0	0.0
7-14-83	781	0.38	78.49	0.0	0.0	2.18	0.70	0.77	0.0
8-12-83	394	12.18	0.0	0.0	0.0	10.91	8.12	2.79	0.0

Effluent Water

Colony	Total	I	II	III	IV	V	VI	VII	VIII
2-12-83	61	4.92	1.64	1.64	11.48	6.56	0.0	*	*
3-14-83	15	20.00	0.0	0.0	0.0	13.33	6.67	*	*
4-13-83	145	3.45	4.14	2.07	0.0	22.07	13.79	0.0	0.0
5-16-83	392	63.78	30.61	0.0	0.0	3.57	1.53	0.0	0.0
6-15-83	152	1.32	1.97	1.31	0.0	8.55	9.87	0.0	0.0
7-14-83	543	0.18	55.25	0.0	0.0	3.32	2.39	2.03	0.0
8-12-83	310	13.23	0.0	0.0	0.0	10.65	8.39	1.94	0.0

Appendix II

Abundance of Certain Bacterial Colony Types Expressed as a Percentage of Total Surface Colonies

POND C

Influent Water

Colony	Total	I	II	III	IV	V	VI	VII	VIII
2-12-83	21	0.0	4.76	4.76	4.76	28.57	0.0	*	*
3-14-83	177	1.13	0.0	1.13	5.65	14.69	10.17	*	*
4-13-83	128	0.78	0.0	0.78	2.34	23.44	14.06	0.0	0.0
5-16-83	95	3.16	0.0	0.0	0.0	24.21	5.26	0.0	0.0
6-15-83	172	4.07	0.0	0.0	0.0	11.05	15.70	0.0	0.0
7-14-83	302	5.30	0.0	0.0	0.0	47.68	1.99	0.0	0.0
8-12-83	294	50.6	0.0	0.0	0.0	22.79	1.70	0.0	0.0

Midwater

Colony	Total	I	II	III	IV	V	VI	VII	VIII
2-12-83	9	44.44	0.0	0.0	0.0	11.11	0.0	*	*
3-14-83	76	17.11	21.05	3.95	2.63	9.21	3.95	*	*
4-13-83	104	8.65	4.81	0.0	0.96	6.73	4.81	0.0	0.0
5-16-83	28	25.00	10.71	0.0	0.0	7.14	17.86	0.0	0.0
6-15-83	215	6.05	0.0	3.72	0.0	7.44	15.81	0.0	0.0
7-14-83	220	1.82	0.0	1.36	0.0	11.36	5.45	15.90	0.0
8-12-83	127	19.69	0.0	0.0	0.0	17.32	17.32	2.36	0.0

Effluent Water

Colony	Total	I	II	III	IV	V	VI	VII	VIII
2-12-83	37	43.24	5.4	0.0	10.81	0.0	0.0	*	*
3-14-83	42	7.14	11.91	2.38	4.76	19.05	4.76	*	*
4-13-83	203	4.93	3.45	0.49	0.0	13.30	9.36	0.0	0.0
5-16-83	30	56.67	10.0	0.0	0.0	6.67	16.67	0.0	0.0
6-15-83	216	3.70	1.85	0.0	0.0	3.24	25.00	0.0	0.0
7-14-83	149	0.67	0.0	3.36	0.0	8.05	14.77	10.74	0.0
8-12-83	158	29.11	0.0	0.0	0.0	15.19	9.49	2.53	0.0

Appendix III

Percent Occurrence of Bacteria Type I

	Pond A					Pond B					Pond C				
	inf	mid	eff	intest	mucous	inf	mid	eff	intest	mucous	inf	mid	eff	intest	mucous
2-12	2.5	7.69	12.70			3.57	0.0	4.92			0.0	44.44	43.24		
3-14	1.16	9.38	22.72	0.0	0.0	2.99	0.0	20.00	0.0	5.00	1.13	17.11	7.14	0.0	7.69
4-13	5.0	4.04	3.94	0.0	2.70	1.37	1.35	3.45	0.0	5.06	0.78	8.65	4.93	0.0	0.0
5-16	5.56	21.05	11.11	0.0	2.55	3.47	54.02	64.78	0.0	8.25	3.16	25.00	56.67	0.0	0.0
6-15	0.84	3.94	6.02	0.0	3.30	3.60	3.80	1.32	0.0	5.00	4.07	6.05	3.70	0.0	0.0
7-14	6.37	3.29	5.21	0.0	3.57	4.39	0.38	0.18	0.0	11.63	5.30	1.82	0.67	0.0	0.0
8-12	20.72	8.23	5.06	0.77	0.0	39.77	12.18	13.23	0.0	0.0	5.06	19.29	29.11	0.0	0.0

Appendix III

Percent Occurrence of Bacteria Type II

	Pond A					Pond B					Pond C				
	inf	mid	eff	intest	mucous	inf	mid	eff	intest	mucous	inf	mid	eff	intest	mucous
2-12	0.0	15.39	1.59			0.0	0.0	1.64			4.76	0.0	5.4		
3-14	0.0	0.0	0.0	0.52	0.0	0.0	0.0	10.00	0.0	0.0	0.0	21.05	11.91	0.0	23.08
4-13	0.63	0.45	0.79	0.0	0.0	0.0	0.67	4.14	0.0	5.06	0.0	4.81	3.45	0.0	5.56
5-16	0.0	0.0	2.78	0.09	0.0	0.0	25.75	30.61	0.0	0.0	0.0	10.71	10.0	0.0	0.0
6-15	0.0	0.0	0.0	0.0	0.0	0.0	5.43	1.97	0.0	0.0	0.0	0.0	1.85	0.0	0.0
7-14	0.0	0.0	1.04	0.0	0.0	0.0	78.49	55.25	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8-12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix III

Percent Occurrence of Bacteria Type III

	Pond A					Pond B					Pond C				
	inf	mid	eff	intest	mucous	inf	mid	eff	intest	mucous	inf	mid	eff	intest	mucous
2-12	10.0	0.0	4.76			5.56	0.0	1.64			4.76	0.0	0.0		
3-14	1.74	3.13	0.0	0.0	0.0	1.20	0.0	0.00	1.02	0.0	1.13	3.95	2.38	0.0	0.0
4-13	0.63	0.45	0.79	0.9	0.0	2.74	1.35	2.07	0.0	0.0	0.78	0.0	0.49	0.59	0.0
5-16	1.23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.03	0.0	0.0	0.0	0.0	0.0
6-15	0.0	1.97	2.26	0.0	0.0	0.0	2.17	1.31	0.0	0.0	0.0	3.72	0.0	0.0	0.0
7-14	0.32	0.76	2.08	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.36	3.36	0.0	0.0
8-12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.49	0.0	0.0	0.0	0.0	0.0	0.0

Appendix III

Percent Occurrence of Bacteria Type IV

	Pond A					Pond B					Pond C				
	inf	mid	eff	intest	mucous	inf	mid	eff	intest	mucous	inf	mid	eff	intest	mucous
2-12	12.5	0.0	12.70			5.36	0.0	11.48			4.76	0.0	10.81		
3-14	5.81	9.38	14.55	2.06	10.71	8.98	0.0	0.00	1.53	5.0	5.65	2.63	4.76	0.0	0.0
4-13	12.5	4.93	0.39	0.0	0.0	2.74	0.0	0.0	0.0	0.0	2.34	0.96	0.0	0.0	0.0
5-16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6-15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7-14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8-12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix III

Percent Occurrence of Bacteria Type V

	Pond A					Pond B					Pond C				
	inf	mid	eff	intest	mucous	inf	mid	eff	intest	mucous	inf	mid	eff	intest	mucous
2-12	10.00	0.0	4.76			3.57	50.00	1.64			28.57	11.11	0.0		
3-14	15.12	28.13	9.09	19.59	17.86	16.17	15.39	13.33	22.65	10.0	14.69	9.21	19.05	10.96	15.39
4-13	16.88	18.83	17.32	7.77	10.81	34.25	27.03	22.07	10.56	18.99	23.44	6.73	13.30	7.7	16.67
5-16	10.49	36.84	19.44	5.56	5.1	18.50	3.45	3.57	11.63	9.28	24.21	7.14	6.67	12.2	1.35
6-15	26.89	16.26	16.54	21.88	3.3	32.43	11.96	8.55	3.57	2.5	11.05	7.44	3.24	20.88	16.67
7-14	43.31	15.95	14.58	5.71	0.0	25.44	2.18	3.32	4.96	2.33	47.68	11.36	8.05	1.38	0.0
8-12	14.36	3.61	7.39	3.08	3.09	18.37	10.91	10.65	0.49	6.67	22.79	17.32	15.19	5.02	3.09

Appendix III

Percent Occurrence of Bacteria Type VI

	Pond A					Pond B					Pond C				
	inf	mid	eff	intest	mucous	inf	mid	eff	intest	mucous	inf	mid	eff	intest	mucous
2-12	0.0	0.0	0.0			0.0	0.0	0.0			0.0	0.0	0.0		
3-14	9.3	12.50	4.55	8.25	17.86	6.59	7.69	6.67	14.25	10.0	10.17	3.95	4.76	13.70	7.69
4-13	15.90	11.66	0.79	26.21	5.41	17.81	15.54	13.79	14.44	10.13	14.06	4.81	9.36	17.16	11.11
5-16	9.26	21.05	8.33	95.49	82.14	7.52	1.15	1.53	77.67	18.56	5.26	17.86	16.67	73.17	68.02
6-15	7.56	14.78	12.78	28.13	23.08	9.01	10.87	9.87	32.74	37.5	15.70	15.81	25.00	29.17	16.67
7-14	2.87	7.85	19.79	38.10	32.14	7.90	0.70	2.39	75.18	32.56	1.99	5.45	14.77	71.56	38.71
8-12	15.90	9.44	10.51	45.38	49.48	4.88	8.12	8.39	38.92	40.00	1.70	17.32	9.49	45.19	49.71

Appendix III

Percent Occurrence of Bacteria Type VII

	Pond A					Pond B					Pond C				
	inf	mid	eff	intest	mucous	inf	mid	eff	intest	mucous	inf	mid	eff	intest	mucous
2-12	*	*	*			*	*	*			*	*	*		
3-14	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
4-13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5-16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6-15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7-14	0.0	10.38	4.17	0.0	0.0	0.0	0.77	2.03	0.71	2.33	0.0	15.90	10.74	0.45	3.23
8-12	15.90	3.01	5.84	1.54	3.09	0.0	2.79	1.94	0.49	0.0	0.0	2.36	2.53	0.84	3.10

Appendix IV

Most Probable Number of Serological Groups of Salmonella Isolated from Water and Fish

Sampling Date		July 28			July 5			July 13		
Influent, pond		A	B	C	A	B	C	A	B	C
Serogroup	B	0.4	-	-	0.9			<0.3	0.3	0.7
	B[1]	0.4	-	-	-			-	-	-
	C-1	-	-	-	-			1.5	0.7	0.9
	C-2	0.4	-	-	-			-	-	-
	C[1]-2	0.9	-	-	-			<0.3	<0.3	0.4
	D-1	-	-	-	-			<0.3	<0.3	<0.3
	E-1	0.4	-	-	-			-	-	-
	E-2	24.0	-	-	-			-	-	-
	H	0.4	-	-	-			-	-	-
Midwater, pond		A	B	C	A	B	C	A	B	C
	B	<0.3	-	-	-	-	-	0.4	<0.3	<0.3
	B[1]	<0.3	-	-	-	-	-	-	-	-
	C-1		-	-	-	0.7	0.9	1.5	0.7	0.9
	C-2	<0.3	-	-	-	-	-	-	-	-
	C[1]-2	0.4	-	-	-	-	-	<0.3	<0.3	<0.3
	D-1		-	-	-	-	-	<0.3	0.9	0.4
	E-1	<0.3	-	-	-	-	-	-	-	-
	E-2	4.3	-	-	-	-	-	-	-	-
	H	<0.3	-	-	-	-	-	-	-	-
Effluent, pond		A	B	C	A	B	C	A	B	C
	B	<0.3	-	-	-	-	-	0.4	0.4	<0.3
	B[1]	0.4	-	-	-	-	-	-	-	-
	C-1		-	-	-	-	-	0.9	0.4	<0.3
	C-2	0.4	-	-	-	-	-	-	-	-
	C[1]-2	0.4	-	-	-	-	-	<0.3	<0.3	<0.3
	D-1	-	-	-	-	-	-	-	-	-
	E-1	<0.3	-	-	-	-	-	-	-	-
	E-2	4.3	-	-	-	-	-	-	-	-
	H	0.4	-	-	-	-	-	-	-	-
Intestine, pond								A	B	C
Serogroup	C-1							<30	70	30

Appendix IV-A

Most Probable Number of Serological Groups of *Salmonella* Isolated from Water and Fish

Sampling Date	July 29			August 5			August 12			Total Number of Isolations
Influent, pond	A	B	C	A	B	C	A	B	C	
Serogroup B	–	–	–	<0.3	<0.3	<0.3	–	–	–	4
B^1	–	–	–	–	–	–	–	–	–	1
C-1	0.9	<0.3	1.5	2.8	0.3	0.7	<0.3	<0.3	<0.3	8
C-2	–	–	–	–	–	–	–	–	–	1
C^1-2	–	–	–	<0.3	<0.3	<0.3	–	–	–	2
D-1	–	–	–	–	–	–	–	–	–	
E-1	–	–	–	–	–	–	–	–	–	1
E-2	–	–	–	–	–	–	–	–	–	1
H	<0.3	<0.3	0.4	–	–	–	–	–	–	2
Midwater, pond	A	B	C	A	B	C	A	B	C	
B	–	–	–	<0.3	<0.3	0.4	–	–	–	2
B^1	–	–	–	–	–	–	–	–	–	
C-1	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	0.4	<0.3	4
C-2	–	–	–	–	–	–	–	–	–	
C^1-2	–	–	–	2.3	0.3	<0.3	–	–	–	3
D-1	–	–	–	–	–	–	–	–	–	1
E-1	–	–	–	–	–	–	–	–	–	
E-2	–	–	–	–	–	–	–	–	–	1
H	<0.3	<0.3	<0.3	–	–	–	–	–	–	
Effluent, pond	A	B	C	A	B	C	A	B	C	
B	–	–	–	–	–	–	–	–	–	2
B^1	–	–	–	–	–	–	–	–	–	1
C-1	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	0.4	0.4	0.9	5
C-2				–	–	–	–	–	–	
C^1-2	–	–	–	4.3	<0.3	<0.3	–	–	–	
D-1	–	–	–	–	–	–	–	–	–	
E-1	–	–	–	–	–	–	–	–	–	2
E-2	–	–	–	–	–	–	–	–	–	
H	<0.3	<0.3	<0.3	–	–	–	–	–	–	
Intestine, pond Serogroup C-1										2

Appendix V

Streptococcus Species Identified from Water and Fish

	Water								
Date	1-83	2-83	3-83	4-83	5-83	6-83	7-83	8-83	Total
Total Number	5	12	12	12	12	12	12	12	89
S. faecalis	3	7	9	9	6	4	8	5	51
S. faccium			1	3	2	1	3	6	16
S. durans	1	4			3				8
S. equinus					1	5			6
S. bovis							1	1	2
Total Group D	4	11	10	12	12	10	12	12	83
S. mitis		1	2			2			5
S. lactis	1								1
Non Group D	1	1	2	0	0	2	0	0	6

Appendix IV

Streptococcus Species Identified from Water and Fish

	Fish Intestine							
Date	1-83	2-83	3-83	4-83	5-83	6-83	7-83	8-83	Total
Total Number		13	13	13	13	13	13	13	91
S. faecalis		9	9	8	8	7	2	3	46
S. faccium			2	5	1	3	3	4	16
S. durans		4					2	3	9
S. equinus			2		4	3			11
S. bovis							6	3	9
Total Group D		13	13	13	13	13	13	13	91
S. mitis									
S. lactis									
Non Group D		0	0	0	0	0	0	0	0

Appendix V

Streptococcus Species Identified from Water and Fish

Fish Mucous									
Date	1-83	2-83	3-83	4-83	5-83	6-83	7-83	8-83	Total
Total Number			4	0	3	0	8		91
S. faecalis			4		2		5		46
S. faccium							2		16
S. durans									9
S. equinus									11
S. bovis					1		1		9
Total Group D			4		3		8		91
S. mitis									
S. lactis									
Non Group D			0	0	0	0	0	0	0